Allozyme frequency changes in two inverse sequences of environments in *Drosophila melanogaster*

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Summary

Six replicates of a *Drosophila melanogaster* population were confronted with 2 sequences of 3 different environments, to wit 3 replicates with the environmental sequence E1-E2-E3 characterizing the H1 history and 3 replicates with the inverse sequence E3-E2-E1 characterizing the H3 history. The 6 replicates maintained in population cages were exposed to each environment for 5 discrete generations. Changes in allozyme frequencies, at 4 loci, were analysed to detect whether the changes depended upon the order of succession of the 3 environments. This was only the case for one locus, *Adh*, but not for *Est-6* and *Pgm*, and can be related to the sensitivity of the *Adh* locus to environmental diversity. For the last locus, α-*Gpdh*, the substantial heterogeneity between replicates within each history seems to be due to a hitchhiking effect.

The diversity of the observed evolutionary profiles, more important between loci for a same history than between the 2 histories for a same locus, seems to point to a set of genetic interactions peculiar to each locus.

Key-words : Environmental diversity, enzyme polymorphism, Drosophila melanogaster, *partition of* χ^2.

Résumé

Variations de fréquences allozymiques dans deux séries inverses d'environnements chez Drosophila melanogaster

Trois répliques d'une population de *Drosophila melanogaster* ont été confrontées à une séquence de 3 environnements différents, caractérisant l'histoire H1. Simultanément, 3 autres répliques de cette population ont été confrontées à la séquence inverse des 3 environnements, caractérisant l'histoire H3. Les 6 répliques ont été gardées, en cage à population, 5 générations discrètes par environnement. A l'aide de la méthode de décomposition du χ^2, les variations de fréquences allozymiques à 4 locus ont été analysées afin de voir si celles-ci dépendaient ou non de l'ordre de succession des 3 environnements. C'est le cas pour le seul locus de l'*Adh*, contrairement à ceux de l'*Est-6* et de la *Pgm*. Ce résultat est mis en relation avec la sensibilité du locus *Adh* à la diversité environnementale. Pour le dernier locus, l'α-*Gpdh*, la grande hétérogénéité des résultats intra-histoire pourrait être due à un effet « hitch-hiking ».

Quant à la diversité des profils évolutifs obtenus, plus grande entre locus pour une même histoire qu'entre les 2 histoires pour un même locus, elle semble témoigner d'un contexte d'interactions géniques particulier à chacun des locus.

Mots clés : Diversité de l'environnement, polymorphisme enzymatique, Drosophila melanogaster, *décomposition du* χ^2.

I. Introduction

In a theoretical article on the « Principle of Historicity in Evolution », LEWONTIN (1967) investigated the pattern of changes in the allelic frequencies of a diallelic locus submitted to 2 reverse sequences of environments. The simulations showed that the pattern of variation of the allelic frequencies over time were different according to the order of the successive environments. Thus, specific selection coefficients for each environment being chosen at random and the allelic frequencies being equal (0.50) at the start of each simulation, the frequency of the reference allele was more often below 0.50 with one of the environmental sequences, more often above 0.50 with the reverse sequence. LEWONTIN emphasized that 2 populations living in precisely the same kind of environment — *i.e.* one in which the probability of selection in a given direction has the same distribution — will nevertheless have totally different life histories.

In the present study we have tested experimentally LEWONTIN's schema using a population of *Drosophila melanogaster* maintained during 5 generations in each of 3 successive environments. The environmental sequence E1-E2-E3 characterized the H1 history and the inverse sequence E3-E2-E1 the H3 history. The variations in allozyme frequencies at 4 loci (α-*Glycerophosphate dehydrogenase, Alcohol dehydrogenase, Esterase-6* and *Phosphoglucomutase*) were followed in order (a) to determine whether or not they were dependent on the order of the 3 successive environments (*i.e.* history) and (b) to analyse the pattern of variations over time (*i.e.* generations).

II. Material and methods

A. *Experimental population*

The population of *D. melanogaster* used in this experiment was SA-FIV. It was constituted in 1979 with 200 pairs of adults derived from a stock obtained, in 1977, from 21 isofemale lines, found to be inversion free (TOKO & CHARLESWORTH, 1982). These isofemale lines were from a population collected, in 1975, by P.T. IVES, in South Amherst, Massachusetts, USA.

B. *Environmental conditions*

Six population cages (36 × 16 × 9 cm plastic boxes) were started, each receiving 2400 adult flies : 3 cages (H1A, H1B, H1C) were used to test the H1 history, *i.e.* the

effect of the environmental succession E1-E2-E3 and 3 others to test the H3 history, *i.e.* the environmental sequence E3-E2-E1. The 6 cages were maintained for 5 discrete generations in each successive environment, whose characteristics were the following :

E1 : discrete 15-day generation, a temperature of 25 °C, a relative humidity (RH) of 50 p. 100. Females allowed to lay eggs during 30 h on 20 vials containing 20 cc of S101 medium (PEARL *et al.*, 1926) with live yeast.

E2 : discrete 25-day generation, 18 °C, RH of 60 p. 100, 3 days for egg laying on 15 vials containing 20 cc of S101 medium with live yeast.

E3 : discrete 25-day generation, 18 °C, RH of 60 p. 100, 3 days for egg laying on 15 vials containing 30 cc of axenic medium — cornmeal 35 g, killed yeast 35 g, Agar 10 g, Nipagine 5 g, water 1 liter (from DAVID, 1959) — supplemented with 100 cc, per liter, of ethanol added at 50 °C and mixed vigorously. The mixture was then poured into vials, stored at 6 °C and used for egg laying from 5 to 6 h later.

As a control, the SA-FIV population was maintained at 18 °C in 12 bottles, mixed at each generation, on cornmeal medium with live yeast. These « keeping » conditions (KC) of the population were supposed to guarantee minimum disturbance for the population.

C. *Electrophoresis*

Electrophoretic assays were conducted on horizontal starch gel (CONNAUGHT) using discontinuous POULIK buffer system (POULIK, 1957). Four polymorphic loci were analysed : α-*Glycerophosphate dehydrogenase, α-Gpdh* (2-20.5) ; *Alcohol dehydrogenase, Adh* (2-50.1) ; *Esterase-6, Est-6* (3-36.8) ; *Phosphoglucomutase, Pgm* (3-43.4). The staining methods were adapted from AYALA *et al.* (1972).

Two allozymes segregate at the α-*Gpdh* ($Gpdh^S$, $Gpdh^F$), *Adh* (Adh^S, Adh^F) and *Est-6* ($Est-6^S = Est-6^{1.00}$, $Est-6^F = Est-6^{1.10}$) loci, and 3 at the *Pgm* locus ($Pgm^S = Pgm^{0.70}$, $Pgm^F = Pgm^{1.00}$, $Pgm^V = Pgm^{1.20}$) (Correspondence for the alleles from OAKESHOTT *et al.*, 1981, 1982).

D. *Statistical analysis*

The data comprise a sequence of allozyme frequencies $\{p_{g,hr}\}$ where $p_{g,hr}$ is the allozyme frequency in the gth generation (g = 0, 1, 5, 6, 10, 11, 15) for the history h (h = 1 for H1, 3 for H3) in the replicate r (r = A, B, C). For 90 p. 100 of the $p_{g,hr}$ values, the estimates were obtained from 150 to 180 adult flies (sex ratio $\simeq$ 1 : 1) collected after the oviposition period.

The variation in allozyme frequencies at a locus was analysed in respect of generations, histories and replicate populations using the partition of χ^2 test (LANCASTER, 1949, 1950 ; IRWIN, 1949). This test provides, for discrete variables, a statistical analysis similar to the test of ANOVA (WINER, 1971), using the observed and expected allelic numbers and not a transformation of the allelic frequencies.

Four factors were considered in the analysis : allele (f), generation (G), history (H) and replicate (R_h) within each history, with α alleles, γ generations, ι histories, ϱ_1 replicates for H1, ϱ_3 replicates for H3 ($\varrho_1 + \varrho_3 = \varrho$). The contingency table

contains α columns and $\gamma \cdot \varrho$ rows. Because the observed and expected marginal totals are fixed, the number of degrees of freedom (d.f.) for the χ^2 of total homogeneity is $\{(\gamma \cdot \varrho) - 1\} \cdot (\alpha - 1)$; the overall value of this total χ^2 was partitioned into additive values of component χ^2 testing the different possible sources of variation of the allozyme frequencies (tabl.2). In this table, these sources of variation of allozyme frequencies of any one locus represent :

Generation effect (G × f) with $(\gamma - 1)(\alpha - 1)$ d.f. : homogeneity of $p_{g...}$ over generations, irrespective of histories and replicates ;

History effect (H × f) with $(\iota - 1)(\alpha - 1)$ d.f. : homogeneity of $p_{..h.}$ over histories irrespective of generations and replicates ;

Interaction Generations × histories (G × H × f) with $(\gamma - 1)(\iota - 1)(\alpha - 1)$ d.f. : homogeneity of $p_{g.h.}$ over generations conditional upon histories irrespective of replicates ;

Replicate effect $(R_1 \times f)$ with $(\varrho_1 - 1)(\alpha - 1)$ d.f. and $(R_3 \times f)$ with $(\varrho_3 - 1)$ $(\alpha - 1)$ d.f. : homogeneity of $p_{..hr}$ over replicates irrespective of generations within both H1 and H3 histories ;

Interaction Generations × replicates $(G \times R_h \times f)$ with $(\gamma - 1)(\varrho_h - 1)(\alpha - 1)$ d.f. : homogeneity of $p_{g.hr}$ over generations conditional upon replicates within both H1 and H3 histories.

In order to itemize, for all generations, the replicate effect within H1 and H3 and the history effect, the component χ^2 testing these effects, generation by generation, were computed (tabl. 3) with $(\varrho_1 - 1)(\alpha - 1)$, $(\varrho_3 - 1)(\alpha - 1)$ and $(\iota - 1)$ $(\alpha - 1)$ d.f. respectively. This 2nd partition of χ^2 was concluded with the computation of the component χ^2 testing the generation effect in each history $(G_h \times f)$ with $(\gamma - 1)(\alpha - 1)$ d.f. When necessary, some other component χ^2 are presented in the text to compare any other particular frequencies.

The formulas used for the calculations of the component χ^2 in a partition were those given by KIMBALL (1954) and MAXWELL (1961).

III. Results

Figures 1 through 4 show the allozyme frequencies observed in each replicate of both histories and table 1 the allelic frequencies of the control population maintained in the « keeping » conditions (KC).

For each locus, the overall χ^2 value is very significant (tabl. 2). The partitioning of these values (tabl. 2 and 3) allowed us to determine the sources of variation causing this high heterogeneity in the allozyme frequencies.

A. *Generation effect* (G × f *and* G_h × f)

The component χ^2 testing the average frequency changes during generations is highly significant for the 4 loci in both histories (tabl. 3). For *ᾱ-Gpdh* and *Est-6* loci, this variation contrasts with the stability observed in the KC (tabl. 1). For *Adh,* the

Adh^F frequency increased in both histories but decreased in the KC, whereas, for *Pgm*, the allelic frequencies changed in a similar manner in both histories and in the KC (*Pgm^F* increased, *Pgm^V* decreased and *Pgm^S* decreased or remained stable).
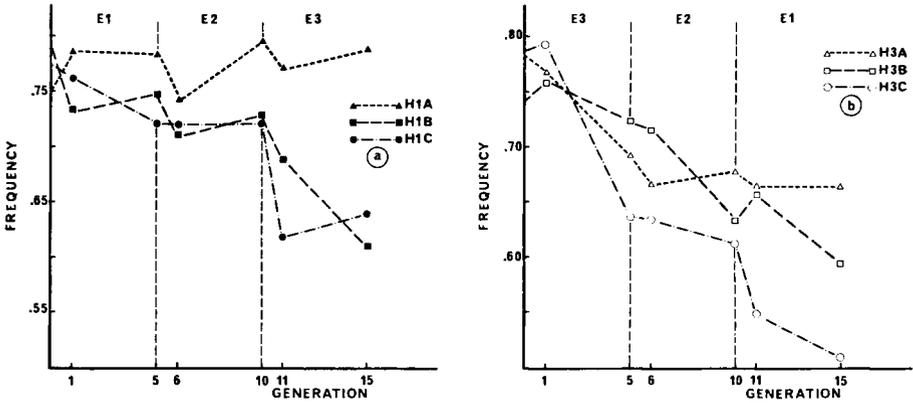

FIG. 1

GpdhF *frequencies for the replicate populations submitted :*
a) to the H1 history (sequence E1-E2-E3 of environments),
b) to the H3 history (sequence E3-E2-E1 of environments).

Fréquences de l'allèle GpdhF *dans les répliques de la population soumises :*
a) à l'histoire H1 (séquence E1-E2-E3 des environnements),
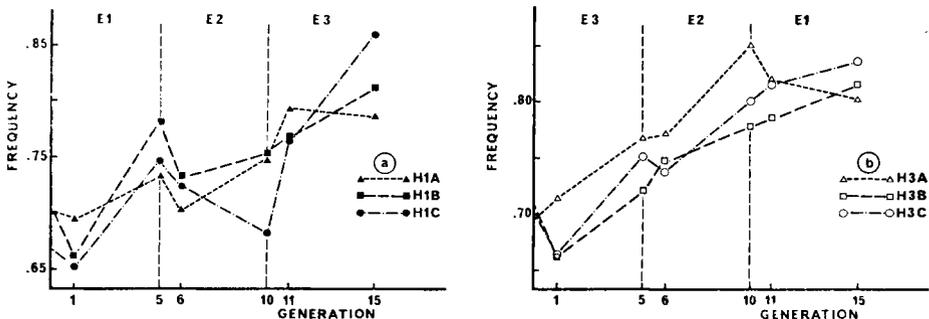*b) à l'histoire H3 (séquence E3-E2-E1 des environnements).*

FIG. 2

AdhF *frequency data : same presentation as in figure 1.*

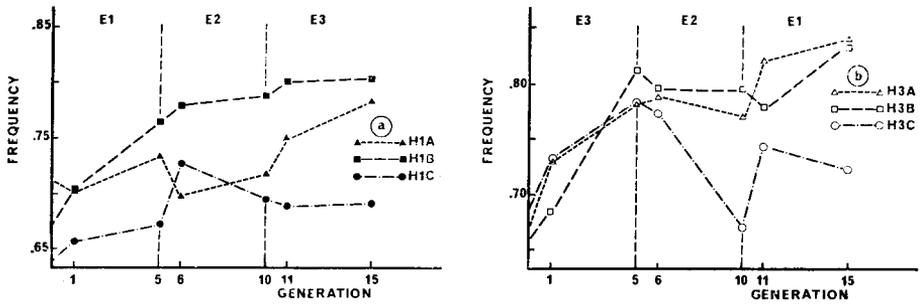*Fréquences de l'allèle* AdhF *: même présentation qu'à la figure 1.*

FIG. 3

Est-6^F *frequency data : same presentation as in figure 1.*

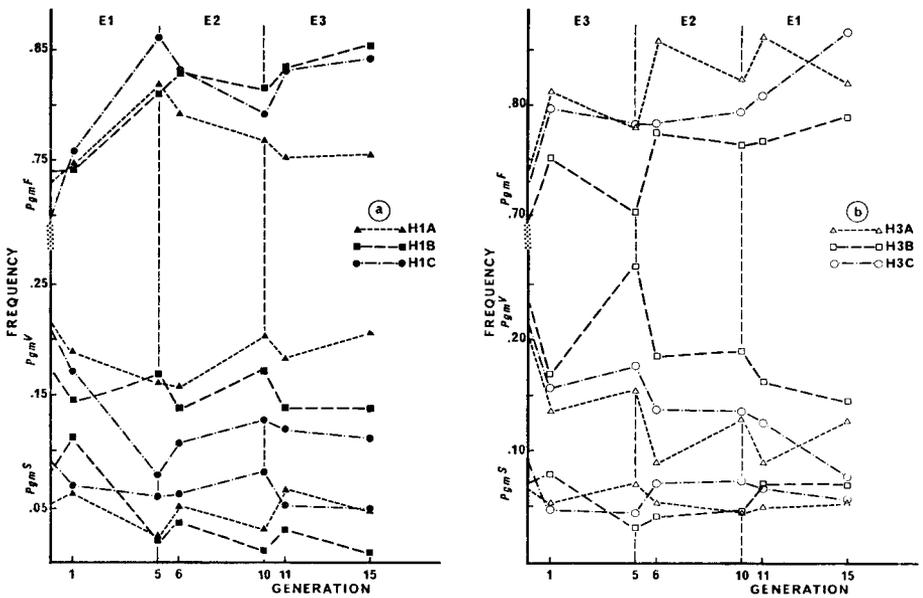*Fréquences de l'allèle* Est-6^F *: même présentation qu'à la figure 1.*

FIG. 4

Pgm^S, Pgm^V and Pgm^F *frequency data : same presentation as in figure 1.*

Fréquences des allèles Pgm^S, Pgm^V *et* Pgm^F *: même présentation qu'à la figure 1.*

B. *Replicate effect* ($R_h \times$ f *and* G $\times R_h \times$ f)

This analysis tests whether the frequency changes from generation to generation were identical between the 3 replicate populations within each history and, conse-

quently, may detect the possible existence of a random drift and its eventual conse-
quences. All loci, except *Adh* (fig. 2), display an intra-history heterogeneity (tabl. 2
and 3).

TABLE 1

Allozyme frequencies for Gpdh^F, Adh^F, Est-6^F, Pgm^V *and* Pgm^F
in the keeping conditions (KC) of the population and χ^2 *of frequency homogeneity.*

Fréquences des allèles Gpdh^F, Adh^F, Est-6^F, Pgm^V *et* Pgm^F
dans les conditions d'entretien (KC) de la population et χ^2 *d'homogénéité des fréquences.*

Generation	Gpdh^F	Adh^F	Est-6^F	Pgm^V	Pgm^F
0	.771 (.014)	.702 (.015)	.672 (.015)	.216 (.014)	.710 (.015)
1	.774 (.013)	.690 (.015)	.674 (.015)	.199 (.013)	.725 (.014)
6	.742 (.025)	.656 (.027)	.707 (.027)	.215 (.024)	.702 (.026)
10	.700 (.026)	.597 (.027)	.641 (.027)	.178 (.021)	.759 (.024)
14	.759 (.025)	.582 (.029)	.643 (.028)	.114 (.018)	.799 (.023)
18	.734 (.025)	.642 (.028)	.661 (.027)	.123 (.019)	.801 (.023)
25	.731 (.025)	.630 (.027)	.630 (.026)	.085 (.015)	.852 (.020)
34	.780 (.025)	.609 (.040)	.716 (.028)	.087 (.017)	.864 (.021)
χ^2	11.38	29.21	9.19		72.35
d.f.	7	7	7		14
	N.S.	p < .001	N.S.		p < .001

Standar errors are in parentheses.
Erreurs standards entre parenthèses.

1) α-*Gpdh* locus (fig. 1) : the replicate effect and the G × R$_h$ × f interaction
are very significant in both histories (tabl. 2). The intra-history heterogeneity started
between generations 10 and 11 (tabl. 3), that is, in the early part of the E3 environ-
ment for the H1 history and of the E1 environment for the H3 history.

For H1, the replicate A in which the $Gpdh^F$ frequency is the highest (fig. 1 a) accounts for all this divergence ($p_{..1B}$ versus $p_{..1C}$: $\chi_1^2 = 0.33$; ns and $p_{..1B+C}$ vs $p_{..1A}$: $\chi_1^2 = 29.11$; p < .001). Moreover, the $Gpdh^F$ frequency did not vary in this replicate ($\chi_6^2 = 3.60$; ns).

For H3, all the divergence is due to the replicate C ($p_{..3A}$ vs $p_{..3B}$: $\chi_1^2 = 1.90$ · ns, and $p_{..3A+B}$ vs $p_{..3C}$: $\chi_1^2 = 18.16$; p < .001) in which the $Gpdh^F$ frequency is the lowest (fig. 1 b).

2) *Est-6* locus (fig. 3) : the replicate effect is significant in both histories (tabl. 2) but the intra-history divergence, manifest from generation 10 onward (tabl. 3), is less pronounced than for α-*Gpdh*.

In H1 (fig. 3 a), the divergence is chiefly due to the replicate C ($p_{..1A}$ vs $p_{..1B}$: $\chi_1^2 = 5.10$; p < .05, and $p_{..1A+B}$ vs $p_{..1C}$: $\chi_1^2 = 12.96$; p < .001) in which the $Est-6^F$ frequency is the lowest. Yet, the absence of significance for the G $\times$ R_1 $\times$ f interaction (tabl. 2) denotes, for this locus, a similar evolutionary profile of the 3 replicates.

For H3 (fig. 3 b), replicate C, in which the $Est-6^F$ frequency is also the lowest, diverged ($p_{..3A}$ vs $p_{..3B}$: $\chi_1^2 = 0.01$; ns, and $p_{..3A+B}$ vs $p_{..3C}$: $\chi_1^2 = 11.19$; p < .001). The heterogeneity between replicates seems greater in H3 than in H1 since the G $\times$ R_3 $\times$ f interaction is significant (tabl. 2).

The origin of the intra-history heterogeneity, for the α-*Gpdh* and *Est-6* loci is likely to be due to the decrease in the population size observed from generations 8 to 10 for H1, from generations 6 to 8 for H3. During these periods the hatching of the eggs and the development of first-instar larvae were rendered difficult by filaments of mould which developed on the surface of the S101 medium vials. Consequently, the number of adult flies emerging in most cages was reduced to about 300-400 individuals. The effective population size may have become small enough to induce a genetic drift that might explain some results obtained from generation 10 onward.

3) *Pgm* locus (fig. 4) : the above hypothesis cannot be the sole explanation for the replicate effect already obvious in generation 5 in both histories (tabl. 3).

During the H1 history, the 3 replicates are heterogeneous ($p_{..1A}$ vs $p_{..1B}$: $\chi_2^2 = 12.00$; p < .01, $p_{..1B}$ vs $p_{..1C}$: $\chi_2^2 = 18.15$; p < .001, and $p_{..1A}$ vs $p_{..1C}$: $\chi_2^2 = 9.73$; p < .01), but as for the α-*Gpdh* locus it is replicate A which shows the larger divergence after generation 10 (fig. 4 a). And the significant G $\times$ R_1 $\times$ f interaction (tabl. 2) confirm this heterogeneity.

During the H3 history the heterogeneity is less pronounced : the G $\times$ R_3 $\times$ f interaction is not significant (tabl. 2) and 2 replicates, A and C, are homogeneous ($\chi_2^2 = 3.78$; ns) whereas replicate C is not ($p_{..3A+C}$ vs $p_{..3B}$: $\chi_2^2 = 29.73$; p < .001). Finally, if the replicate effect is significant for generations 5 and 6, this is no longer the case from generation 10 onward (tabl. 3).

TABLE 2

Partition of total frequency homogeneity χ² for each locus.

Décomposition du χ² total d'homogénéité des fréquences pour chaque locus.

Source of variation	Value of component χ²				d.f. †
	αGpdh	Adh	Est-6	Pgm	
Generation effect : G × f	144.4***	158.0***	91.7***	97.8***	6
History effect : H × f	53.5***	8.6**	23.3***	5.2	1
Interaction : G × H × f	35.1***	13.5*	6.0	32.5**	6
H1 : Replicate effect : R_1 × f	29.4***	1.4	35.4***	41.3***	2
Interaction : G × R_1 × f	32.7**	16.1	15.6	46.4**	12
H3 : Replicate effect : R_3 × f	20.1***	4.3	11.2***	33.5***	2
Interaction : G × R_3 × f	29.2**	8.5	23.8*	32.4	12
Total	344.4***	210.4***	207.0***	289.1***	41

* $p < .05$ - ** $p < .01$ - *** $p < .001$.

† : The number of d.f. is twice for *Pgm*.

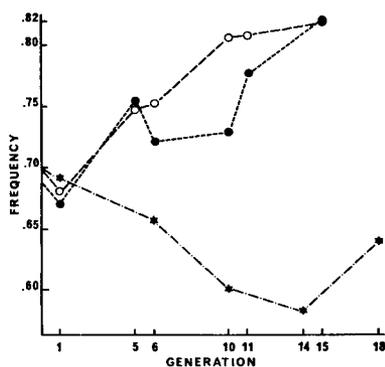*Le nombre de d.d.l. est double pour le locus* Pgm.

FIG. 5

Changes in AdhF *mean frequency during the H1 history* $(p_{g,1})$ *(●),*
the H3 history $(p_{g,3})$ *(○), and in the keeping conditions (★) of the SA-FIV population.*

Variations de la fréquence moyenne de l'allèle AdhF *durant l'histoire H1* $(p_{g,1})$ *(●),*
l'histoire H3 $(p_{g,3})$ *(○), et dans les conditions d'entretien (★) de la population SA-FIV.*

Value, for each locus, of component χ^2 corresponding to : — replicate effect (2 d.f.) in the H1 and H3 histories for each generation (g), — history effect (1 d.f.) for each generation (g), — generation effect (6 d.f.) in the H1 $(G_1 \times f)$ and H3 $(G_3 \times f)$ histories.

Valeur, pour chaque locus, des χ^2 constitutifs correspondant à : — l'effet réplique (2 d.d.l.) à chaque génération (g) dans les histoires H1 et H3. — l'effet histoire (1 d.d.l.) à chaque génération (g), — l'effet génération (6 d.d.l.) dans les histoires H1 $(G_1 \times f)$ et H3 $(G_3 \times f)$.

	αGpdh	Adh	Est-6	Pgm †
Replicate effect				
H1 : g = 0	1.4	1.5	4.5	6.1
1	1.9	1.7	2.2	7.5
5	2.9	1.9	6.9*	17.3*
6	0.8	0.8	5.6	5.3
10	5.0	5.2	7.9*	21.7***
11	19.0***	0.9	10.4**	9.7
15	31.1***	5.4	13.3**	19.9***
H3 : g = 0	1.7	0.2	0.1	2.4
1	1.0	2.7	2.2	5.4
5	5.6	1.8	0.7	19.6***
6	4.9	1.0	0.5	13.4**
10	3.4	4.8	10.6**	9.2
11	14.5***	1.3	5.4	8.7
15	18.2***	1.1	14.8***	7.2
History effect				
g = 0	0.0	0.4	0.2	0.9
1	0.4	0.2	1.8	3.8
5	9.6**	0.1	11.8***	16.7***
6	6.2*	2.4	6.5*	0.5
10	25.6***	16.2***	1.2	2.2
11	15.6***	2.7	2.7	3.3
15	20.9***	0.0	5.3*	10.4**
Generation effect				
$G_1 \times f$	35.2***	82.6***	29.8***	63.8***
$G_3 \times f$	144.3***	88.9***	67.9***	66.5***

* p < .05 - ** p < .01 - *** p < .001.

† : The number of d.f. is twice for *Pgm*.

  Le nombre de d.d.l. est double pour le locus Pgm.

C. History effect (H × f and G × H × f)

The analysis of this effect shows whether the changes in allozyme frequency depended upon the order of succession of the 3 environments. The results are different for the four loci.

1) *Adh* locus (fig. 5): though in generation 15 the average frequency is identical between the 2 histories ($p_{15,1.} = 0.820$ and $p_{15,3.} = 0.818$), the Adh^F frequency behavior appears to depend upon the succession of environments. Thus, the history effect is significant as well as the G × H × f interaction (tabl. 2). The generation by generation comparison (tabl. 3)shows that the history effect is due to a divergent response to the E2 environment. This environment was experienced by the flies after 5 generations in the E1 or in the E3 environment with similar mean frequencies ($p_{5,1.} = 0.754$ and $p_{5,3.} = 0.746$). However, after 5 generations in the E2 environment (*i.e.* at generation 10), the Adh^F mean frequency has remained stable in the H1 history ($p_{10,1.} = 0.727$, $\chi^2_1 = 1.79$; ns) but has increased in the H3 history ($p_{10,3.} = 0.806$, $\chi^2_1 = 9.19$; p < .01).

The Adh^F frequency behavior differed whether the population experienced the H1 or the H3 history.

2) *α-Gpdh* locus : the history effect is very significant as well as the G × H × f interaction (tabl. 2). This inter-history divergence is conspicuous after 5 generations (tabl. 3). However, on account of the important intra-history heterogeneity, it is difficult to conclude that after generation 6 the history effect is not due to random drift. Indeed if we consider the pairs of homogeneous replicates, H1B and H1C on the one hand and H3A and H3B on the other, the total history effect is just significant at 5 p. 100 ($\chi^2_1 = 4.02$), whereas the G × H × f interaction ($\chi^2_6 = 6.25$; ns) and the frequency difference in generation 15 ($\chi^2_1 = 0.01$; ns) are not. The appearance of a history effect is due to the divergent replicates : H1A, in which the $Gpdh^F$ frequency is the highest (fig. 1 a), H3C, in which this frequency is the lowest (fig. 1 b).

3) *Est-6* locus : if the global history effect is manifest, the lack of significance for the G × H × f interaction (tabl. 2) denotes an equivalent evolutionary behavior during both histories. The frequency changes seem to be associated with the maintenance of the flies in population cages, a state which constituted a constant factor during the 15 generations of each history. Nevertheless the effect of the E1 and E3 environment appears different for the first 5 generations (tabl. 3).

4) *Pgm* locus : the absence of significance for the total history effect contrasts with the significance of the G × H × f interaction (tabl. 2). This contrast seems to denote a random nature of the frequency changes. The hypothesis is further supported by the fact that in generation 6 the history effect vanished at the same time as the replicate effect in the H1 history (tabl. 3) and reappeared in generation 15 when the replicate effect became again very important in H1.

IV. Discussion

The study, in the SA-FIV population, of the allozyme frequency behavior in temporally varied environments assigned to verify this behavior in constant environmental conditions and with the least possible competition (KC). This seemed particularly important since the population arrived only a short time before the experiments began. Thus (tabl. 1) a frequency stability was registered for α-*Gpdh* and *Est-6* loci, while for *Adh*, and even more for *Pgm*, the frequencies varied in a manner that does not seem to be apparently random considering the results of the first 2 loci.

Within both histories, the comparisons of the replicate populations revealed that the temporal observed changes of allozyme frequencies were due in part to random drift. Indeed all loci except *Adh* displayed within both histories a significant replicate effect. But, depending on the locus, the cause of the intra-history divergence seems to have neither the same origin nor the same consequences. In both histories, from generation 10 onward, this divergence is very substantial for α-*Gpdh* but moderate for *Est-6*. As for *Pgm*, the divergence appeared as early as generation 5, remaining substantial after generation 10 in the H1 history but disappearing after this generation in the H3 history.

Genetic drift is responsible for 2 outcomes. Primarily, the simple random variations of frequencies affecting all loci. Secondarily, linkage disequilibrium which, if joined with a hitchhiking effect (MAYNARD-SMITH & HAIGH, 1974) can amplify the effects of genetic drift on some loci. On account of, for α-*Gpdh*, the great observed heterogeneity and the diversity of the divergent replicates from generation 10 onward, the hypothesis of a hitchhiking effect having affected the α-*Gpdh* frequency changes can be suggested. The consequences of this effect can have varied between replicates according to the importance of the disequilibrium established between the α-*Gpdh* locus and another locus, under selection, tightly linked to it.

An understanding of the relationship between genetic variations at loci encoding enzymes and environmental variations is decisive to any discussion of the role of natural selection in evolution. But actually there is only a small amount of experimental evidence supporting the hypothesis that environmental variation is tracked by allozyme frequencies (HEDRICK *et al.*, 1976). In *Drosophila,* the main laboratory experiments have investigated the effects of spatial environmental variation (HALEY & BIRLEY, 1983). In contrast, we have studied the point with respect to the temporal environmental variation. The present results credit a positive response of the *Adh* locus to environmental conditions. These results include : the frequency homogeneity, contrary to the 3 other loci, observed between replicates within both histories ; the increase in *Adh^F* frequency in both histories as opposed to its decrease in the keeping conditions ; and finally the sensitivity to the order of the succession of the 3 environments (fig. 5). The sensitivity of this locus to environmental diversity has been previously reported by VAN DELDEN *et al.* (1978). The principal factors of variation (and perhaps, consequently, of selection) in our environments were the temperature, the nutritive value of the larval culture medium, its humidity level and the addition, or not, of ethanol. And the action of selection on the *Adh* frequencies by factors such as temperature (McKENZIE & McKECHNIE, 1981 ; VIGUE *et al.*, 1982), humidity (VAN DELDEN *et al.*, 1978) and presence of ethanol (OAKESHOTT & GIBSON, 1981) has been claimed several times, if not demonstrated unambiguously.

The results observed at the 3 remaining loci seem to be independent of both environmental diversity and of the order of environmental succession. For *Pgm*, there is no difference between the 2 histories irrespective of generations and the evolutionary behavior is similar in all experiments (H1, H3 and KC). For *α-Gpdh*, the appearance of an effect due to the order of the environmental sequence cannot be accepted since it results from an important intra-history divergence, obvious in both histories and probably due to a hitchhiking effect. In addition, such a hitchhiking effect may be the unique cause of the frequency changes in both histories in contrast with the stability registered in the keeping conditions. As for *Est-6* in contrast to the stability observed in the keeping conditions, both histories induce a frequency variation, but this variation is independent of the sequence of environments. It seems simply to be associated with the maintenance of the flies in population cages.

In conclusion, the diversity of evolutionary profiles observed in our experiments seems much more important between the 4 loci within the same history than between the 2 histories for the same locus. This suggests that such diversity of the evolutionary profiles results more from the peculiar context of each locus in the genome than from the environmental diversity. It is possible that different kinds of interactions between each locus under consideration and other units of the genome have exerted an impact (as is certainly the case for the *α-Gpdh* locus). This aspect of the eventual role of interacting polymorphic loci on allozyme frequency changes is further broached by MERÇOT (1985).

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